# PATENT COOPERATION TREATY

# PCT



## INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

(Chapter II of the Patent Cooperation Treaty)

(PCT Article 36 and Rule 70)

| | | |
|---|---|---|
| Applicant's or agent's file reference<br>32733PC01 | **FOR FURTHER ACTION** | See Form PCT/IPEA/416 |
| International application No.<br>PCT/DK2004/000263 | International filing date *(day/month/year)*<br>07.04.2004 | Priority date *(day/month/year)*<br>08.04.2003 |
| International Patent Classification (IPC) or national classification and IPC<br>G01N33/574 | | |
| Applicant<br>COLOTECH A/S et al. | | |

1. This report is the international preliminary examination report, established by this International Preliminary Examining Authority under Article 35 and transmitted to the applicant according to Article 36.
2. This REPORT consists of a total of 7 sheets, including this cover sheet.
3. This report is also accompanied by ANNEXES, comprising:
   a. ☒ *sent to the applicant and to the International Bureau)* a total of 3 sheets, as follows:
      ☒ sheets of the description, claims and/or drawings which have been amended and are the basis of this report and/or sheets containing rectifications authorized by this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions).
      ☐ sheets which supersede earlier sheets, but which this Authority considers contain an amendment that goes beyond the disclosure in the international application as filed, as indicated in item 4 of Box No. I and the Supplemental Box.
   b. ☐ *(sent to the International Bureau only)* a total of (indicate type and number of electronic carrier(s)) , containing a sequence listing and/or tables related thereto, in computer readable form only, as indicated in the Supplemental Box Relating to Sequence Listing (see Section 802 of the Administrative Instructions).

4. This report contains indications relating to the following items:

| | | |
|---|---|---|
| ☒ | Box No. I | Basis of the opinion |
| ☐ | Box No. II | Priority |
| ☒ | Box No. III | Non-establishment of opinion with regard to novelty, inventive step and industrial applicability |
| ☐ | Box No. IV | Lack of unity of invention |
| ☒ | Box No. V | Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement |
| ☐ | Box No. VI | Certain documents cited |
| ☐ | Box No. VII | Certain defects in the international application |
| ☒ | Box No. VIII | Certain observations on the international application |

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| Date of submission of the demand<br>22.02.2005 | Date of completion of this report<br>31.05.2005 |
| Name and mailing address of the international preliminary examining authority:<br>European Patent Office - P.B. 5818 Patentlaan 2<br>NL-2280 HV Rijswijk - Pays Bas<br>Tel. +31 70 340 - 2040 Tx: 31 651 epo nl<br>Fax: +31 70 340 - 3016 | Authorized Officer<br>Gundlach, B<br>Telephone No. +31 70 340-4478 |

# INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
PCT/DK2004/000263

## Box No. I Basis of the report

1. With regard to the **language**, this report is based on the international application in the language in which it was filed, unless otherwise indicated under this item.

   ☐ This report is based on translations from the original language into the following language , which is the language of a translation furnished for the purposes of:
   - ☐ international search (under Rules 12.3 and 23.1(b))
   - ☐ publication of the international application (under Rule 12.4)
   - ☐ international preliminary examination (under Rules 55.2 and/or 55.3)

2. With regard to the **elements*** of the international application, this report is based on *(replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report)*:

| | |
|---|---|
| **Description, Pages** | |
| 1-87 | as originally filed |
| **Claims, Numbers** | |
| 1-11 | received on 24.02.2005 with letter of 22.02.2005 |
| **Drawings, Sheets** | |
| 1/20-20/20 | as originally filed |

   ☐ a sequence listing and/or any related table(s) - see Supplemental Box Relating to Sequence Listing

3. ☒ The amendments have resulted in the cancellation of:
   - ☐ the description, pages
   - ☒ the claims, Nos. 12-32
   - ☐ the drawings, sheets/figs
   - ☐ the sequence listing *(specify)*:
   - ☐ any table(s) related to sequence listing *(specify)*:

4. ☐ This report has been established as if (some of) the amendments annexed to this report and listed below had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).
   - ☐ the description, pages
   - ☐ the claims, Nos.
   - ☐ the drawings, sheets/figs
   - ☐ the sequence listing *(specify)*:
   - ☐ any table(s) related to sequence listing *(specify)*:

* *If item 4 applies, some or all of these sheets may be marked "superseded."*

# INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
PCT/DK2004/000263

**Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability**

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

☐ the entire international application,

☒ claims Nos. 1,2 (both partially)

because:

☒ the said international application, or the said claims Nos. 1,2 (both partially) relate to the following subject matter which does not require an international preliminary examination (specify):
**see separate sheet**

☐ the description, claims or drawings *(indicate particular elements below)* or said claims Nos. are so unclear that no meaningful opinion could be formed *(specify)*:

☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

☐ no international search report has been established for the said claims Nos.

☐ the nucleotide and/or amino acid sequence listing does not comply with the standard provided for in Annex C of the Administrative Instructions in that:

| | | |
|---|---|---|
| the written form | ☐ | has not been furnished |
| | ☐ | does not comply with the standard |
| the computer readable form | ☐ | has not been furnished |
| | ☐ | does not comply with the standard |

☐ the tables related to the nucleotide and/or amino acid sequence listing, if in computer readable form only, do not comply with the technical requirements provided for in Annex C-*bis* of the Administrative Instructions.

☐ See separate sheet for further details

# INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
PCT/DK2004/000263

## Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

| | | | |
|---|---|---|---|
| Novelty (N) | Yes: | Claims | 1-11 |
| | No: | Claims | |
| Inventive step (IS) | Yes: | Claims | 1-11 |
| | No: | Claims | |
| Industrial applicability (IA) | Yes: | Claims | 3-11 |
| | No: | Claims | |

2. Citations and explanations (Rule 70.7):

**see separate sheet**

## Box No. VIII Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

**see separate sheet**

## Re Item I

**Basis of the report**

Amendments made are considered not to go beyond the disclosure as filed.

## Re Item III

**Non-establishment of opinion with regard to novelty, inventive step and industrial applicability**

Claims 1 and 2 do not exclude *in vivo* use of the marker(s) in question. Therefore, claims 1 and 2 at least partially relate to subject-matter considered by this Authority to be covered by the provisions of Rule 67.1(iv) PCT. Consequently, no opinion will be formulated with respect to the industrial applicability of the subject-matter of these claims (Article 34(4)(a)(I) PCT).

## Re Item V

**Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**

None of the documents cited describes a marker for colorectal cancer having an apparent molecular weight of 3980 Da. All claims are therefore considered to be novel.

D6 is considered to represent the closest prior art and discloses:
An in vitro method of diagnosing colorectal cancer in a sample from a mammal, the method comprising:
assaying said sample by a quantitative detection assay and determining the intensity signal of at least one marker (claim 43) when assaying a serum sample with SELDI-TOF mass spectrometry (page 9, lines 12-16) using protein chips from Ciphergen Biosystems (page 9, line 29- page 10, line 13). The difference between present claim 1 and D6 is the disclosure of a specific marker having a molecular weight of 3980 Da. There seems to be no unexpected effect derivable from this difference. The objective problem to be solved can therefore be formulated as relating to: How to provide a further marker for colorectal cancer? The solution, use of the marker having 3980 Da is considered to be inventive for the following reasons: There is no suggestion in the prior art cited that such a marker

exists. Accordingly, presence of inventive step can be acknowledged.

Subject-matter of claims 3-11 is industrially applicable.

**Re Item VIII**

**Certain observations on the international application**

In the present claims the markers in question are characterised by the "**apparent** molecular weight" in Daltons. This implies that a precise value is not needed for the person skilled in the art in order to understand the limits of the claimed scope.

However, from the list of suitable markers it appears as if some of the markers were closely related to each other in their molecular weight (e.g. the marker of claim 1 having an apparent molecular weight of 3980 Da and one of the markers of claim 2 having 3984 Da apparent molecular weight). These closely related markers imply that the **precise** molecular weight is indeed crucial for the definition of the claimed scope since otherwise a person skilled in the art would not be able to carry out the invention. This need of the precise molecular weight represents a contradiction in the light of the language used (see above). Accordingly, the claims cannot be considered to fulfil the requirements of Article 6 PCT as they are not clear.

Furthermore, in order to identify the precise markers methods must be used which allow to determine the molecular weight with a preciseness of at least 1 Da (the marker of 3980 Da differs from that with 3984 Da only by 4 Da).

Accordingly, also claim 7 cannot be considered to fulfil the requirements of Article 6 PCT since methods involving e.g. 2D gels are not suitable for determination of the molecular weight with such a precision.

It is not clear whether the international application discloses a way to determine the molecular weight with such a precision. In other words it is doubtful whether the invention is disclosed in a manner being sufficiently clear and complete to be carried out by a person skilled in the art (Article 5 PCT).

It is clear from e.g. the description on page 16 that the following feature is essential to the definition of the invention:

"**The intensity signal for marker [...] 3980 Da is decreased** when assaying a serum

sample on IMAC30 chip (Ciphergen)." Without that feature the invention claimed could not be carried out. If the signal is decreased in serum samples of a patient it must also be present in serum samples of healthy persons. Therefore, a measurement of a decrease of the signal (with a certain threshold) is essential to the invention.

Since independent claims 1 and 3 do not contain this feature they do not meet the requirement following from Article 6 PCT taken in combination with Rule 6.3(b) PCT that any independent claim must contain all the technical features essential to the definition of the invention.

## Claims

1. Use of a polypeptide marker having the apparent molecular weight of 3980 Da for the prediction of the clinical outcome, complications and mortality of an individual diagnosed with colorectal cancer.

2. Use of a polypeptide marker according to claim 1 in combination with at least one additional marker selected from the group consisting of the polypeptides having apparent molecular weights of

66800 Da, 66500 Da, 66300 Da, 64860 Da, 60730 Da, 60500 Da, 60475 Da, 46000 Da, 45500 Da, 44300 Da, 33000 Da, 28040 Da, 28025 Da, 28010 Da, 28000 Da, 27700 Da, 19966 Da, 19900 Da, 19865 Da, 16150 Da, 15935 Da, 15580 Da, 15200 Da, 15140 Da, 14470 Da, 14300 Da, 14100 Da, 14030 Da, 13870 Da, 13747 Da, 11723 Da, 13700 Da, 13331 Da, 13265 Da, 12000 Da, 11989 Da, 11987 Da, 11900 Da, 11700 Da, 11650 Da, 11550 Da, 11500 Da, 11133 Da, 11080 Da, 10830 Da, 9950 Da, 9700 Da, 9600 Da, 9197 Da, 9140 Da, 9090 Da, 9079 Da, 8971 Da, 8940 Da, 8931 Da, 8930 Da, 8652 Da, 8580 Da, 8230 Da, 7469 Da, 7324 Da, 7023 Da, 6880 Da, 6850 Da, 6660 Da, 6650 Da, 6635 Da, 6450 Da, 6436 Da, 6435 Da, 6430 Da, 6125 Da, 6110 Da, 6090 Da, 5920 Da, 5906 Da, 5905 Da, 5900 Da, 5871 Da, 5857 Da, 5540 Da, 5360 Da, 5330 Da, 5266 Da, 5260 Da, 5234 Da, 5075 Da, 4977 Da, 4749 Da, 4660 Da, 4640 Da, 4634 Da, 4500 Da, 4480 Da, 4460 Da, 4330 Da, 4300 Da, 4290 Da, 4281 Da, 4270 Da, 4266 Da, 4264 Da, 4168 Da, 4136 Da, 4039 Da, 4024 Da, 4000 Da, 3984 Da, 3960 Da, 3895 Da 3882 Da, 3878 Da, 3816 Da, 3777 Da, 3712 Da, 3680 Da, 3651 Da, 3574 Da, 3570 Da (def 2), 3487 Da, 3480 Da (def 3),3450 Da (def 1),3444 Da, 3408 Da, 3372 Da, 3280, 3275 Da, Da, 3160, Da, 2960 Da, 2955 Da, 2933 Da, 2878 Da, 2850 Da, 2840 Da, 2799 Da, 2693 Da, 2462 Da, 2450 Da, 2364 Da, 2330 Da, 2275 Da, 2230 Da, 2210 Da, 1945 Da,1930 Da, 1688 Da, 1536 Da, 1365 Da, 1256 Da, 1042 Da, 1026 Da, and 1005 Da,

for the prediction of the clinical outcome, complications and mortality of an individual diagnosed with colorectal cancer.

3. A method of diagnosing colorectal cancer in a sample from a mammal, the method comprising

assaying a sample obtained from said mammal by a quantitative detection assay and determining the intensity signal of a marker having apparent molecular weight of 3980 Da

comparing said intensity signal with a reference value

identifying whether the intensity signal of the marker from the sample is significantly different from the reference value.

4. A method according to claim 3, wherein the marker having the molecular weight of 3980 Da is combined with at least one additional marker selected from the group consisting of the polypeptides having apparent molecular weights of

66800 Da, 66500 Da, 66300 Da, 64860 Da, 60730 Da, 60500 Da, 60475 Da, 46000 Da, 45500 Da, 44300 Da, 33000 Da, 28040 Da, 28025 Da, 28010 Da, 28000 Da, 27700 Da, 19966 Da, 19900 Da, 19865 Da, 16150 Da, 15935 Da, 15580 Da, 15200 Da, 15140 Da, 14470 Da, 14300 Da, 14100 Da, 14030 Da, 13870 Da, 13747 Da, 11723 Da, 13700 Da, 13331 Da, 13265 Da, 12000 Da, 11989 Da, 11987 Da, 11900 Da, 11700 Da, 11650 Da, 11550 Da, 11500 Da, 11133 Da, 11080 Da, 10830 Da, 9950 Da, 9700 Da, 9600 Da, 9197 Da, 9140 Da, 9090 Da, 9079 Da, 8971 Da, 8940 Da, 8931 Da, 8930 Da, 8652 Da, 8580 Da, 8230 Da, 7469 Da, 7324 Da, 7023 Da, 6880 Da, 6850 Da, 6660 Da, 6650 Da, 6635 Da, 6450 Da, 6436 Da, 6435 Da, 6430 Da, 6125 Da, 6110 Da, 6090 Da, 5920 Da, 5906 Da, 5905 Da, 5900 Da, 5871 Da, 5857 Da, 5540 Da, 5360 Da, 5330 Da, 5266 Da, 5260 Da, 5234 Da, 5075 Da, 4977 Da, 4749 Da, 4660 Da, 4640 Da, 4634 Da, 4500 Da, 4480 Da, 4460 Da, 4330 Da, 4300 Da, 4290 Da, 4281 Da, 4270 Da, 4266 Da, 4264 Da, 4168 Da, 4136 Da, 4039 Da, 4024 Da, 4000 Da, 3984 Da, 3960 Da, 3895 Da 3882 Da, 3878 Da, 3816 Da, 3777 Da, 3712 Da, 3680 Da, 3651 Da, 3574 Da, 3570 Da (def 2), 3487 Da, 3480 Da (def 3),3450 Da (def 1),3444 Da, 3408 Da, 3372 Da, 3280, 3275 Da, Da, 3160, Da, 2960 Da, 2955 Da, 2933 Da, 2878 Da, 2850 Da, 2840 Da, 2799 Da, 2693 Da, 2462 Da, 2450 Da, 2364 Da, 2330 Da, 2275 Da, 2230 Da, 2210 Da, 1945 Da,1930 Da, 1688 Da, 1536 Da, 1365 Da, 1256 Da, 1042 Da, 1026 Da, and 1005 Da.

5. A method according to claim 3 or 4, wherein the reference value(s) is/are intensity signal value(s) calculated from data of said marker(s) obtained from a sample without colorectal cancer from the same mammal.

6. A method according to claim 3 or 4, wherein the reference value(s) is/are intensity signal value(s) calculated from data of said marker(s) obtained from samples from at least one normal mammal.

7. A method according to any of claims 3-6, wherein the quantitative detection assay is selected from the group consisting of immunoassay, kinetic/real-time PCR, 2D gel, protein array, gene array and other nano-technology methods.

8. A method according to any of claims 3-7, wherein the signal is selected from the group consisting of fluorescence signal, mass spectrometry images, radioactivity and enzyme activity.

9. A method according to any of claims 3-8, wherein the intensity signal for the at least one additional marker 15200 Da, 6125 Da, 5900 Da, 3275 Da and 2955 Da is increased and the intensity signal for markers 4290 Da, 2450 Da, and 1536 Da is decreased when assaying a serum sample on a protein chip that incorporates carboxylate chemistry that acts as a weak cation exchanger, such as a CM10 protein chip.

10. A method according to any of claims 3-8, wherein the intensity signal for the additional markers 33000 Da, 16150 Da, 15935 Da, and 15200 Da is increased when assaying a serum sample on a protein chip being a strong anion exchange array with quaternary amine functionality, such as a Sax2protein chip.

11. A method according to claim 3-8, wherein the intensity signals for the additional markers 5340 Da and 5906 Da is increased and the intensity signals for markers 6880 Da and 28010 is decreased when assaying a serum sample on a having an immobilized metal affinity capture array with a nitriloacetic acid (NTA) surface, such as a IMac30 chip.